Research Article

Simultaneous Analysis and Validation of Ciprofloxacin-Diclofenac and Tetracycline-Diclofenac by Using UV Spectrophotometer

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ABSTRACT

The present study was aimed to develop a simple and precise UV Spectrophotometric method for simultaneous estimation of ciprofloxacin -Diclofenac and tetracycline- Diclofenac in chitosan dental films developed for treatment of periodontics. The Spectrophotometric methods using chemo metric techniques were followed for the simultaneous determination of ciprofloxacin /tetracycline and Diclofenac sodium using their zero order spectra. Chemo metric method is classical least square (CLS) Inverse least square (ILS) Principle component regression (PCR) Partial least square (PLS) calibrations. Multilevel multifactor design was in the range of 2-10 µg mL^{-1} for ciprofloxacin and 4-32 µg mL^{-1} for Diclofenac in ciprofloxacin/Diclofenac combination. Similarly, the levels were in the range of 4-32 µg mL^{-1} for both tetracycline and Diclofenac in case of tetracycline/Diclofenac combination. UV spectra were recorded in the wavelength range 230-310 nm versus solvent blank and digitized absorbance was recorded at 1 nm intervals. The computation was made in R-software environment. CLS, PCR and PLS algorithms were applied to the UV absorption data matrix of these binary mixtures to determine calibration equations. The predictive ability of a calibration is the standard error of calibration (SEC) and prediction (SEP) And Relative standard error (RSE %). Factor 6 and 5 was optimum for the estimation of principle ingredients by PLS and PCR in CIPRO and DICLO, TETRA and DICLO combination. The maximum values of the mean percent error were found to be smallest, good recovery values and the relative standard deviation of our developed method was acceptable. Precision repeatability and intermediate precision measurement trials were in acceptable range indicating good precision of the proposed method. The results of proposed method have been validated as per ICH guidelines. The results of analysis of correlation values for all the drugs were shown high precision, less expensive than other methods.

Keywords: Ciprofloxacin-Diclofenac, UV spectrophotometry method, Tetracycline-Diclofenac.

INTRODUCTION

Periodontal diseases are a general term which encompasses several pathological conditions affecting the tooth supporting structures. It includes conditions such as chronic periodontitis, aggressive periodontitis, systemic disease-associated periodontitis and necrotizing periodontitis.[1] These conditions are characterized by a destruction of the periodontal ligament, a resorbtion of the alveolar bone and the migration of the junctional epithelium along the tooth surface.

The aim of current periodontal therapy is to remove the bacterial deposits from the tooth surface and to shift the pathogenic microbiota to one compatible with periodontal health. Therapeutic approaches include mechanical scaling and root planning and in some cases surgery. As a result of treatment, there is a decrease of gingival inflammation as well as clinical probing depth.[2]

Ciprofloxacin is a quinolone group of agent with a bactericidal in action and acts by inhibiting DNA synthesis. It has broad spectrum of antibacterial activity including Gram negative aerobic bacteria and very sensitive against β-lactamase producing strains. Ciprofloxacin is also effective against periodontal pathogens including *Actinobacillus actonomycetumcomitans, Prevotella intermedia, Porpheromonas gingivalis* etc.[3]

Tetracycline's are primarily bacteriostatic antimicrobials, effective against all Gram positive and many Gram negative bacteria and exert their antibacterial activity by inhibiting microbial protein synthesis.[3]

Diclofenac has analgesic, antipyretic and anti-inflammatory activities. It is a non-selective cyclooxygenase inhibitor and its potency is greater than other NSAIDs. In addition, Diclofenac appears to reduce intracellular concentration of free arachidonate in leucocytes, perhaps by altering the release or uptake of the fatty acid.[4]

To estimate the drug content and release studies of combination profile of the formulations, the analytical method which involves simultaneous estimation of the components was developed. Multivariate calibration methods are chemo metric tools that are applied to the analysis of Spectrophotometric data. The Spectrophotometric methods using chemo metric techniques were followed for the simultaneous determination of ciprofloxacin /tetracycline and Diclofenac sodium using their zero order spectra.[5] High-performance liquid chromatography has become an important tool for routine determination of antimicrobial agents in biological fluids. Many chromatographic methods for the determination of fluoroquinolone and several analytical methods for the quantification of DICLO in biological fluids have been reported with the majority

based on different extraction procedures and coupled to chromatographic systems. In the past decade, multivariate techniques have been incorporated into analytical methodologies.[6-8]

The Process Analytical Technology (PAT) initiative has introduced a number of rapid spectroscopic methods of analysis to the pharmaceutical, chemical, food and related industries, but associated with these methods is a large amount of data to be interpreted. Applications, such as Near Infrared (NIR) Spectroscopy requires statistical methods to be applied to extract the relevant information out of the data. The data analysis methods are collectively referred to as Chemo metrics. The principles of Chemo metrics however extend beyond its application to spectroscopic data, for example, analyzing chromatographic, electrometric data etc.[9] This is a rapid spectroscopic tool to handle large data. Applying the principles of chemo metrics can lead to development of a powerful, simple, comprehensive system for characterizing measurement reliability of analytical processes.

MATERIALS AND METHODS

A drug such as Ciprofloxacin was obtained from Dr.Reddy's laboratories, Hyderabad, Tetracycline from K.A.P.L., Bangalore and Diclofenac Sodium from Eros Pharmaceuticals, Bangalore. All the other chemicals used in this study are of analytical reagent grade. In this work, an attempt was made to develop Spectrophotometric method for the simultaneous determination of ciprofloxacin/tetracycline and Diclofenac sodium using multivariate calibration technique.

The Process Analytical Technology (PAT) initiative has introduced a number of rapid spectroscopic methods of analysis to the pharmaceutical, chemical, food and related industries, but associated with these methods is a large amount of data to be interpreted. Applications, such as Near Infrared (NIR) Spectroscopy requires statistical methods to be applied to extract the relevant information out of the data. The data analysis methods are collectively referred to as Chemo metrics. The principles of Chemo metrics however extend beyond its application to spectroscopic data, for example, analyzing chromatographic, electrometric data etc.[9] This is a rapid spectroscopic tool to handle large data. Applying the principles of chemo metrics can lead to development of a powerful, simple, comprehensive system for characterizing measurement reliability of analytical processes.

The chemo metric generally presumed that there is a linear relationship between digitized data and component concentration. These methods have a calibration step followed by validation (with validation samples independently prepared) and prediction (new samples) step in which the results of calibration step are used to estimate the component concentration from unknown sample spectrum.[10-15]

There are four chemo metric methods, namely:

A) Classical least squares (CLS)

B) Inverse least squares (ILS)

C) Principle component regression (PCR) and

D) Partial least squares (PLS) calibrations.

Chemo metric techniques are other methods gaining wide application for the resolution of the drug mixtures. A calibration set of 16 samples were randomly prepared in aqueous acetic acid (1% v/v), by multilevel multifactor design in which four levels of concentrations of CIPRO and DICLO/ TETRA and DICLO were introduced. The levels were in the range of 2-10 µg mL^{-1} for CIPRO and 4-32 µg $mL^{-1}$for DICLO in ciprofloxacin/Diclofenac combination. Similarly, the levels were in the range of 4-32µg mL^{-1} for both TETRA and DICLO in case of tetracycline/Diclofenac combination, and illustrated in Table 3.2.

UV spectra were recorded in the wavelength range 230-310 nm

The predictive ability of a calibration model in chemo metric methods can be defined in various ways. The most general expression is the standard error of calibration (SEC) and prediction (SEP), which is given by the following equation;

$$\text{SEP} \quad (\text{SEC}) \quad = \sqrt{\frac{\sum_{i=1}^{N} (C_i^{Added} - C_i^{Found})^2}{n}}$$

Here C_i^{Added} is the added concentration of drugs, C_i^{Found} is the predicted concentration of drugs and n is the total number of the synthetic mixtures.

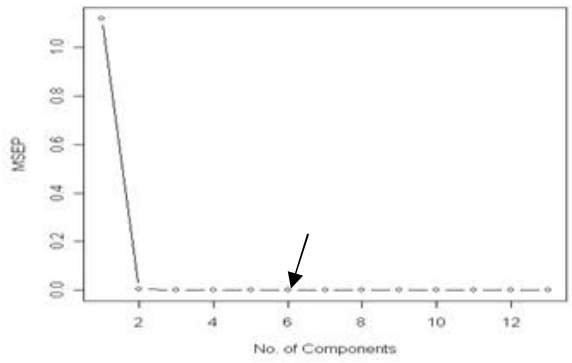
RESULTS AND DISCUSSION

Rapid, precise, accurate, specific and simple UV Spectrophotometry using three chemo metric calibrations namely, Partial least square (PLS), principal component regression (PCR) and classical least square (CLS) were developed for the simultaneous determination of CIPRO/DICLO and TETRA/DICLO from combined film formulations. In these methods using chemo metric techniques, the calibrations were constructed by using the absorption data matrix, with measurements in the range of 230-310 nm (Δλ = 1 nm) in their zero order spectra and calibrations were realized by using R – software (version 2.1.1). The validity of the proposed methods was successfully assessed for analysis of both drugs in laboratory prepared mixtures and in developed film formulations.

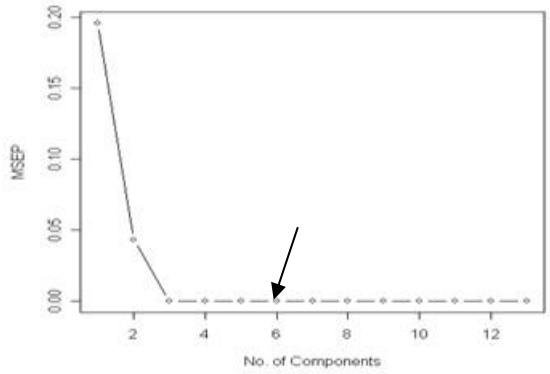
For PCR and PLS methods, 16 calibration spectra were used for the selection of the optimum number of factors by using the cross validation technique. This allows modeling of the system with the optimum amount of information and avoidance of over fitting or under fitting. The cross-validation procedure consisting of systematically removing one of a group of training

samples in turn and using only the remaining ones for the construction of latent factors and applied regression. The predicted concentrations were then compared with the actual ones for each of the calibration samples and mean squares error of prediction (MSEP) was calculated. The MSEP was computed in the same manner each time a new factor was added to the PCR and PLS model. The selected model was that with the fewest number of factors such that its MSEP values were not significantly greater than that for the model, which yielded the lowest MSEP. A plot of MSEP values against number of components, Figure 1 and 2 indicates that factor6 was optimum for the estimation of principle ingredients by PLS and PCR in CIPRO and DICLO combination. Similarly, Figure 3 and 4 indicates that factor 5 was optimum for the estimation of principle ingredients by PLS and PCR in case of TETRA and DICLO combination. At the selected principal components of PLS and PCR the concentrations of each sample were then predicted and compared with known concentration and the PRESS (prediction error sum of squares) was calculated. It was given by this equation, and values are indicated in Table 1.

$$\text{PRESS} = \sum_{i=1}^{n} \left(C_i^{Added} - C_i^{Found} \right)^2$$

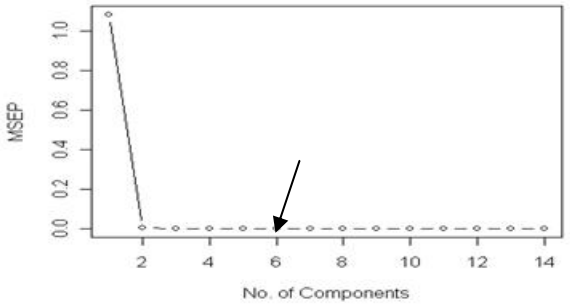


A

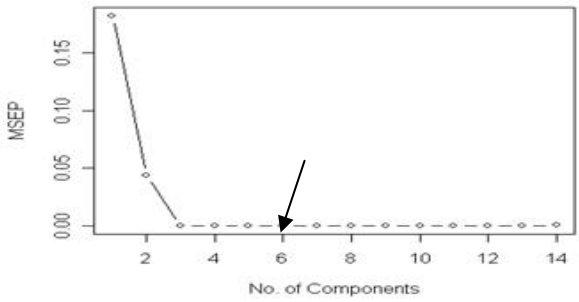


B

Figure 1: MSEP plots of a calibration set obtained using leave-one-out (LOO) cross validation of PLS-model for A) CIPRO and B) DICLO in zero-order absorption data.

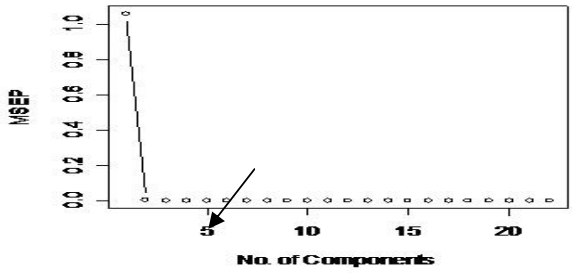


A

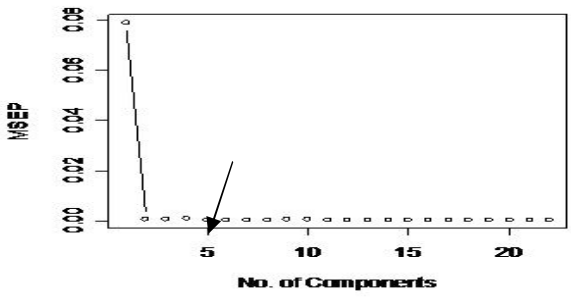


B

Figure2. MSEP plots of a calibration set obtained using leave-one-out (LOO) cross validation of PCR model for A) CIPRO and B) DICLO in first derivative absorption data.



A



B

Figure 3: MSEP plots of a calibration set obtained using leave-one-out (LOO) cross validation of PLS-model for A) TETRA and B) DICLO in zero-order absorption data.

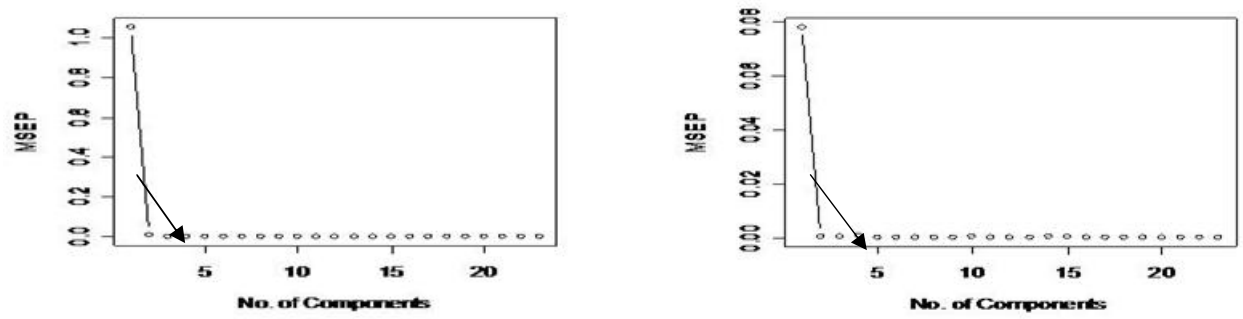


Figure 4: MSEP plots of a calibration set obtained using leave-one-out (LOO) cross validation of PCR-model for A) TETRA and B) DICLO in zero order absorption data

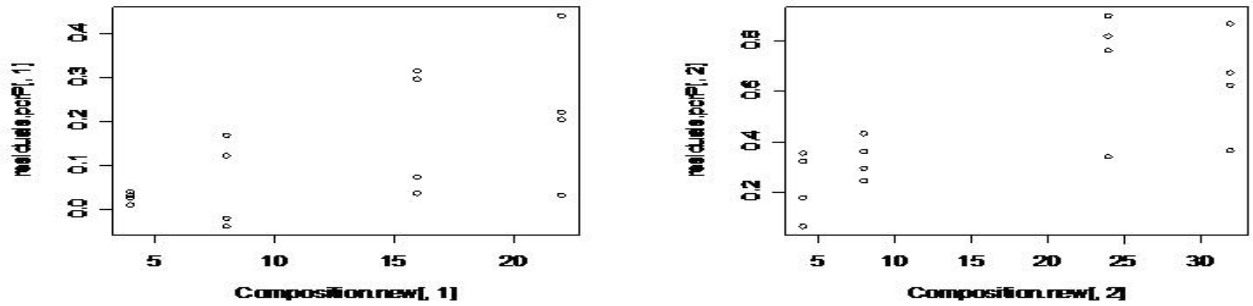


Figure 5: Plot of concentrations residuals (residuals pcrP) against the predicted concentrations (Composition new) of A) TETRA and B) DICLO in prediction set.

Table 1: Statistical parameters of chemo metric methods in prediction step of Zero- order spectra

Component		CIPRO	DICLO	TETRA	DICLO
CLS	*SEP*	0.1573	0.2472	1.8660	0.3541
	A	0.0551	0.2551	1.6477	0.4823
	B	1	0.9813	0.9444	0.9842
	R	0.9996	0.9996	0.9990	0.9997
PCR	*SEP*	0.1108	0.1495	0.5550	0.1879
	A	0.0529	0.0535	0.1988	0.0239
	B	1.0003	0.9988	1.0161	1.0116
	R	0.9998	0.9998	0.9998	0.9998
PLS	*SEP*	0.1114	0.1495	0.5495	0.1768
	A	0.0478	0.0535	0.182	0.034
	B	1.0007	0.9988	1.0166	1.0115
	R	0.9998	0.9998	0.9998	0.9998

a: Intercept; *b*: slope; *r*: correlation coefficient.

Validation of the Developed Method

To check the validity (predictive ability) of the calibration models, the simultaneous analysis of the prediction set containing16 samples of various concentrations (in triplicates) of CIPRO and DICLO as well as TETRA and DICLO was carried outand the results are given in Table 4.7 and 4.8. The maximum values of the mean percent errors corresponding to CLS, PCR and PLS for the same mixtures were completely acceptable because of their very smallest values. The mean recoveries and the relative standard deviations of our developed methods were computed and indicated in Table 4.7.and 4.8. Their numerical values were completely acceptable because of their good recovery values and hence found satisfactory for the validation.

Table 2: Recovery results in prediction from Zero- order spectra for CIPRO and DICLO in synthetic mixtures by proposed chemo metric techniques

Mixture added		Recovery (%)						Error%					
		CLS		PLS		PCR		CLS		PLS		PCR	
Cipro	Diclo	Cipro	Diclo	Cipro	Diclo	Cipro	Diclo	Cipro	Diclo	Cipro	Diclo	Cipro	Diclo
2	4	98.83	109.7	100.7	98.85	100.6	98.88	-1.17	9.78	0.67	-1.15	0.61	-1.12
2	12	98.31	104.8	100.7	100.3	100.5	100.2	-1.69	4.82	0.65	0.28	0.53	0.29
2	24	110.2	100.1	99.5	98.9	99.34	98.88	10.24	0.18	-0.47	-1.1	-0.66	-1.12
2	32	104.3	100.6	99.97	100	99.84	100.0	4.36	0.65	-0.03	0.01	-0.16	0
4	4	98.37	100.7	100.9	100.6	100.9	100.5	-1.63	0.76	0.94	0.56	0.86	0.56
4	12	100.7	106.9	101.2	97.85	101.2	97.87	0.74	6.96	1.23	-2.15	1.22	-2.13
4	24	101.6	103.5	101.3	100.2	101.3	100.2	1.69	3.58	1.28	0.24	1.3	0.25
4	32	101.9	100.8	101.2	100.1	101.2	100.1	1.98	0.89	1.23	0.11	1.2	0.11
8	4	101.7	97.81	102.0	97.58	102.1	97.59	1.78	-2.19	2.04	-2.42	2.12	-2.41
8	12	100.0	99.33	101.6	99.48	101.7	99.49	0.07	-0.67	1.62	-0.52	1.66	-0.51
8	24	99.94	103.4	100.4	96.41	100.4	96.42	-0.06	3.44	0.44	-3.59	0.41	-3.58
8	32	99.63	100.8	100.1	98.61	100.1	98.61	-0.37	0.84	0.07	-1.39	0.06	-1.39
10	4	98.55	98.61	99.09	98.34	99.05	98.34	-1.45	-1.39	-0.91	-1.66	-0.95	-1.66
10	12	100.2	99.64	101.0	100.1	101.0	100.1	0.2	-0.36	1.03	0.11	1.04	0.11
10	24	100.2	98.95	100.7	99.64	100.7	99.64	0.2	-1.05	0.68	-0.36	0.68	-0.36
10	32	99.96	102.8	100.2	98.31	100.1	98.32	-0.04	2.82	0.15	-1.69	0.12	-1.68
	$\bar{x}$	100.7	100.9	100.6	99.21	100.6	99.21	--	--	--	--	--	--
	RSD	2.5	3.2	0.8	1.2	0.8	1.2	--	--	--	--	--	--

$\bar{x}$, Mean recovery value; RSD, Relative standard deviation

Table 3: Recovery results in prediction from Zero- order spectra for TETRA and DICLO in synthetic mixtures by proposed chemo metric techniques

Mixture added		Recovery (%)						Error%					
		CLS		PLS		PCR		CLS		PLS		PCR	
Tetra	Diclo	Tetra	Diclo	Tetra	Diclo	Tetra	Diclo	Tetra	Diclo	Tetra	Diclo	Tetra	Diclo
4	4	99.87	102.69	101.37	100.79	101.52	101.01	- 0.13	2.69	1.37	0.79	1.52	1.01
4	12	103.28	105.87	107.86	102.02	107.99	102.11	3.28	5.87	7.86	2.02	7.99	2.11
4	24	101.36	106.39	103.79	101.68	104.34	101.85	1.36	6.39	3.79	1.68	4.34	1.85
4	32	105.09	104.58	107.03	101.85	107.84	102.00	5.09	4.58	7.03	1.85	7.84	2.00
12	4	100.08	104.59	103.55	99.95	103.65	100.21	0.08	4.59	3.55	-0.05	3.65	0.21
12	12	100.03	102.11	102.91	101.32	103.05	101.51	0.03	2.11	2.91	1.32	3.05	1.51
12	24	102.54	98.89	105.44	101.97	105.40	101.96	2.54	-1.11	5.44	1.97	5.40	1.96
12	32	101.65	99.67	104.11	100.86	104.48	101.00	1.65	-0.33	4.11	0.86	4.48	1.00
24	4	99.32	103.54	101.37	100.26	101.41	100.63	-0.68	3.54	1.37	0.26	1.41	0.63
24	12	99.99	101.68	103.75	99.69	103.75	99.72	-0.01	1.68	3.75	-0.31	3.75	-0.28
24	24	98.98	98.37	103.20	100.49	103.17	100.45	-1.02	-1.63	3.20	0.49	3.17	0.45
24	32	100.03	97.98	103.36	100.87	103.40	100.93	0.03	-2.02	3.36	0.87	3.40	0.93
32	4	98.35	97.93	102.65	100.19	102.71	100.81	-1.65	-2.07	2.65	0.19	2.71	0.81
32	12	97.98	100.26	101.87	99.10	101.95	99.50	-2.02	0.26	1.87	-0.90	1.95	-0.50
32	24	99.89	103.58	102.23	100.51	102.10	100.22	-0.11	3.58	2.23	0.51	2.10	0.22
32	32	101.78	100.98	101.09	100.03	101.13	100.14	1.78	0.98	1.09	0.03	1.13	0.14
	$\bar{x}$	100.64	101.82	103.47	100.88	103.62	100.88	--	--	--	--	--	--
	RSD	1.86	2.75	1.87	0.86	1.98	0.82	--	--	--	--	--	--

$\bar{x}$, Mean recovery value; RSD, Relative standard deviation.

Another diagnostic test for chemo metric methods was carried out by plotting the concentrations residuals against the predicted concentrations. The residuals appear randomly distributed around zero, indicating good prediction ability of the model as shown in Figure 5.

Linearity and range

The linearity of the proposed chemo metric method for determination of drugs was evaluated by analyzing a series of different concentrations of standard drug. In this study six concentrations were chosen, ranging between 2-32 µg $ml^{-1}$for both the drugs. Each concentration was repeated three times and obtained information on the variation absorbances at stated wavelength region. The linearity of the calibration graphs was validated by the high value of correlation coefficient, slope and the intercept value.

Precision

Method reproducibility

Method reproducibility was demonstrated by repeatability and intermediate precision measurements of recoveries for each title ingredient. The repeatability (within-day in triplicates) and intermediate precision (for 3 days) was carried out at five concentration levels for each compound. The obtained results within and between days trials were in acceptable range indicating good precision of the proposed method (Table 4 and 5).

Table 4: Precision study results of prepared binary mixture of CIPRO and DICLO

Validation parameter	Chemo metric		
	% recovery RSD		
Repeatability[a]	CLS	PCR	PLS
CIPRO	2.035	2.057	1.487
DICLO	1.697	1.234	1.954
Intermediate precision[b]			
CIPRO	2.032	1.304	1.971
DICLO	1.9874	1.647	1.654

[a]Repeatability, three replicates of five concentration levels within-day; [b]Intermediate precision, three replicates of five concentration levels between-days (3-days).

Table 5: Precision study results of prepared binary mixture of TETRA and DICLO

Validation parameter	Chemo metric		
	% recovery RSD		
Repeatability[a]	CLS	PCR	PLS
TETRA	2.0850	1.8144	1.834
DICLO	2.1625	0.3159	0.3269
Intermediate precision[b]			
TETRA	2.4462	1.0867	1.5971
DICLO	1.9874	0.9346	0.2906

[a]Repeatability, three replicates of five concentration levels within-day; [b]Intermediate precision, three replicates of five concentration levels between-days (3-days).

Table 6: Results obtained for the prepared film samples by using chemo metric calibrations.

Methods	% Recovery			Methods	% Recovery		
	CLS	PCR	PLS		CLS	PCR	PLS
TD-I				**CD-I**			
TETRA mean[a] ± SD[b]	99.4 ± 1.31	99.3 ± 0.98	99.3 ± 0.93	**CIPRO** mean[a] ± SD[b]	101.13 ± 0.495	100.01± 0.773	100.78 ± 0.377
DICLO mean[a] ± SD[b]	99.0 ± 0.99	100.1 ± 0.17	101.4 ± 0.44	**DICLO** mean[a] ± SD[b]	99.52± 1.521	101.74± 1.531	101.74 ± 1.531
TD-II				**CD-II**			
TETRA mean[a] ± SD[b]	99.2 ± 1.16	101.2 ± 0.38	101.3 ± 2.10	**CIPRO** mean[a] ± SD[b]	100.17± 0.659	100.2 ± 0.48	101.2 ± 1.21
DICLO mean[a] ± SD[b]	99.0 ±1.03	99.4 ± 0.93	100.5 ± 1.97	**DICLO** mean[a] ± SD[b]	102.11± 1.4	99.5 ± 0.97	101.2 ± 1.99
TD-III				**CD-III**			
TETRA mean[a] ± SD[b]	99.1 ± 1.13	100.2 ± 0.37	101.4 ± 2.11	**CIPRO** mean[a] ± SD[b]	99.12 ± 2.13	101.2 ± 1.37	100.4 ± 1.11
DICLO mean[a] ± SD[b]	100.2 ± 1.98	100.0 ± 0.27	101.3 ± 0.42	**DICLO** mean[a] ± SD[b]	101.2 ± 1.88	100.1 ± 0.17	101.5 ± 0.47

Theoretical Drug loading: TD-I/CD-I: Tetracycline/Ciprofloxacin 20 % and Diclofenac Sodium 10 % per film**;** TD-II/CD-II: Tetracycline/ Ciprofloxacin 15 % and Diclofenac Sodium 15 % per film**;** TD-II/ CD-III: Tetracycline/Ciprofloxacin 10 % and Diclofenac Sodium 20 % per film**;** a, Mean recovery value of five determinations for each method. b, Standard deviation; ($n_1 = n_2 = 5$), Theoretical values for *t* and F at P = 0.05 are 2.31 and 6.39 respectively.

Results of Analysis of Formulations

The experimental results of the proposed methods on developed film formulations were presented in Table 6. The results of all methods were very close to each other as well as to the value of theoretical drug loading in the films. The statistical tests denote no significant difference in the results achieved by all three methods. The numerical values of all statistical parameters indicate that the investigated methods are suitable for the determination of both the drugs in formulations.

CONCLUSION

The developed method was free from interferences due to excipients present in formulation and it can be used for routine quality control analysis. The present research study proved that chemo metric techniques developed for simultaneous estimation of ciprofloxacin /Diclofenac and Tetracycline/Diclofenac demonstrated high reproducibility with good recovery and robustness in various prepared combination film formulations. The results of analysis of correlation values for all the drugs were shown high precision. The proposed methods are

less expensive than other methods hence it can be applied for the routine analysis of these drugs in the commercial formulations without any pretreatment and without any prior separation step.

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